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ORIGINAL ARTICLE

Evaluation of sperm motility across varying thawing temperatures in Jersy, Holstein Friesian, and Murrah Bulls

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Abstract

Background: The heating rate during the thawing of frozen semen significantly impacts the functional activation of mitochondria, which affects sperm motility assessment.

Methods: This study examined the effect of thawing temperature on the motility of spermatozoa in cryopreserved semen. A total of 240 semen straws (0.25 ml each) from 24 bulls (8 Jersey, 8 Holstein Friesian (HF), and 8 Murrah breed), aged 2 to 6 years, were used. Semen was collected, analyzed, processed, frozen, and stored in liquid nitrogen using a standard protocol with a tris-citrate-egg yolk extender. Samples were thawed for 30 seconds in a water bath at temperatures T1: 32°C, T2: 34°C, T3: 36°C, T4: 38°C, and T5: 40°C. Sperm motility, progressive motility, fast motility, slow motility, and immotile percentage of the frozen semen were evaluated. A computer-assisted semen analyzer (CASA) was used for analysis. The data was entered into MS-Excel and analyzed using analysis of variance (ANOVA), with the significance between treatments assessed using Duncan's multiple range test (DMRT) post hoc test.

Results: Jersey semen showed the best motility percentage at T3 ($p<0.05$) (T1: 53.025±6.73, T2: 60.01±3.81, T3: 74.33±1.40, T4: 65.59±3.17, T5: 59.92±3.58). HF semen also showed the best motility percentage at T3 ($p<0.05$) (T1: 60.67±6.31, T2: 55.93±6.31, T3: 76.6±2.28, T4: 72.93±2.10, T5: 57.69±2.28). Murrah semen showed the best motility percentage at T4 ($p<0.05$) (T1: 56.63±3.93, T2: 58.58±4.34, T3: 77.09±1.59, T4: 82.72±4.03, T5: 72.87±4.00). Progressive motility (%) was highest at T3 for Jersey (52.41±2.97), T3 for HF (56.29±4.65), and T4 for Murrah (63.94±6.37) ($p<0.05$). Fast motility (%) was highest at T3 for Jersey (12.71±2.04), T3 for HF (18.43±1.63), and T4 for Murrah (12.589±2.74) ($p<0.05$). Slow motility (%) was lowest at T3 for Jersey (27.8±3.43), T3 for HF (34.23±2.72), and T4 for Murrah (31.32±4.72) ($p<0.05$). Immotility (%) was lowest at T3 for Jersey (25.73±1.37), T3 for HF (23.4±2.28), and T4 for Murrah (17.27±4.03) ($p<0.05$).

Conclusion: Thawing at T3 (36°C) enhances motility, progressive motility, and fast motility while reducing slow motility and immotile percentage in Jersey semen. Similarly, thawing at T4 (38°C) improves motility, progressive motility, and fast motility while reducing slow motility and immotile percentage in Murrah semen.

Keywords: Thawing, Semen analysis, Conception rate, Reproduction failures, Artificial insemination, cattle.

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Introduction

Artificial insemination (AI) is a technique in which spermatozoa are collected from a male, diluted with a suitable extender, and then transferred into the cervix or uterus of a female to achieve conception. Sperm must meet minimum seminal standards, including concentration, motility, and viability for the success of insemination. After collection and processing, semen is storage under optimum conditions and thawed, during insemination at the right temperature and loaded into a sterilized AI gun for insemination. (Nagata *et al*. 2019).

The global demand for food and quality protein has steadily increased in recent decades, with livestock serving as a primary source of milk, meat, and other nutritional needs. To meet this demand, high-producing animals are essential, and their productivity is largely determined by efficient breeding programs and sound management practices. A significant factor affecting cost effectiveness of livestock productivity is fertility. Reproductive failures is resulting from poor fertilizing ability of spermatozoa, inadequate semen preservation (Lessard *et al*. 2000). It involves cooling spermatozoa to subzero temperatures using liquid nitrogen at -196°C, allowing for the long-term preservation of sperm cells (Lieberman *et al*. 2016), Most effective method for preserving semen, allowing for long-term storage of viable sperm cells and the propagation of genetic material. This advancement has facilitated genetic improvements, such as sperm sexing, embryo freezing, and superovulation (Holt, 2000; Kaabi *et al*. 2003). Improper handling of semen during transportation, storage, and exposure to environmental conditions further compromises sperm viability. These issues have resulted in lower reproductive performance, particularly in cattle. As a solution, AI offers an economical and effective way to introduce superior genetics into the herd without the need for bull rearing, which is costly and impractical for small-scale farmers. The practice reduces the risk of inbreeding and enhances productivity, especially in remote areas where access to high-quality bulls is limited (Hansen, 2009).

Semen cryopreservation is essential for the long-term storage of spermatozoa. However, faulty cryopreservation technique may adversely affect sperm motility, primarily due to mitochondrial damage, which is crucial for energy production and function (Schuster *et al*. 2003). The freezing and thawing processes might also alter the sperm membranes, reducing motility and fertilizing potential (Holt 2000). Cryoprotectants, although essential for preventing ice formation during freezing, is also responsible to induce oxidative stress, which impairs sperm activity and motility (Longobardi *et al*. 2020, Wang *et al*. 2014). Despite its advantages, cryopreservation can result in a reduction in motility and viability due to sperm membrane damage and oxidative stress (Roca *et al*. 2006). Sperm motility is fundamental for AI success and assessing the motility for selecting high-quality sperm for fertilization.

The study is aimed to investigate the effects of different thawing temperatures on spermatozoa motility, focusing on progressive motility, fast motility, slow motility, and immobility. The null hypothesis posits that thawing temperature has no effect on sperm motility, while the alternate hypothesis suggests a significant impact. This research will provide valuable insights into how thawing temperatures influence sperm quality and help optimize AI practices, ensuring better reproductive outcomes.

Materials and methods

Study site

The study was conducted at the Frozen Semen Station, National Livestock Breeding Office (NLBO), Pokhara, Nepal (83°58’20.604’’E, 28°15’48.996’’N, 793 m elevation), with an average temperature of 22°C and 570 mm rainfall.

Sperm motility across varying thawing temperatures

Sampling

The semen samples were collected from the diseased free bulls. A total of 240 frozen semen straws (0.25 ml each) were collected from 24 sexually mature bulls (8 Jersey, 8 Holstein Friesian, 8 Murrah), aged 2 to 6 years.

Animals and their management

All bulls were housed in the well-ventilated with Fresh Napier (Pennisetum spp.) and Teosinte (Zee mays subsp. parviglumis) fodder were provided to bulls, in addition to food supplements and trace mineral supplements. The supply of fresh water was ad libitum. Two times a week, the bull was thoroughly exercised and groomed before his semen was collected. Average body weight of Jersey is 720 Kilogram (K.G) (n=8) with history of sire milk 10000 liter/lactation and Dam milk 4800 liter/lactation, meanwhile HF average weight is 850 K.G (n=8) with history of sire milk 10000 liter/lactation and Dam 12500 liters/lactation, Similarly, Murrah average weights is 580 K.G, with history of sire milk 3200 liter/lactation and Dam milk 3150 liter/lactation. The artificial vaginal (AV) with Warm water 37°C. was used to collect semen.

Routine evaluation of semen

Routine examination of semen was quantitative and qualitative examination. Quantitative methods are volume, viscosity, presence of blood or external debris, meanwhile qualitative methods are spermatozoa concentration, initial sperm motility. Only semen straws meeting the minimum standard of NLBO were used for analysis.

Motility Test Procedure

Motility evaluation was performed using AndroVision® CASA (Minitube GmbH, Germany). Ten fields per sample were assessed, and the mean result was recorded. Semen analysis was performed using the Computer-Assisted Semen Analyzer (CASA) Androvision (Minitube GmbH, Germany). The resulting data were collected and analyzed to assess the motility characteristics of the spermatozoa at different thawing temperatures.

Experimental design

The frozen semen straws were thawed for 30 seconds in five treatment groups as follows:
Group A: Frozen semen straw thawed at 32 °C
Group B: Frozen semen straw thawed at 34 °C
Group C: Frozen semen straw thawed at 36 °C
Group D: Frozen semen straw thawed at 38 °C, and
Group E: Frozen semen straw thawed at 40 °C.

Results and Discussion

A total of 24 bulls (8 Jersey, 8 Holstein Friesian, and 8 Murrah) were selected for the study.

Effects of thawing temperature on spermatozoa motility factor of different bull

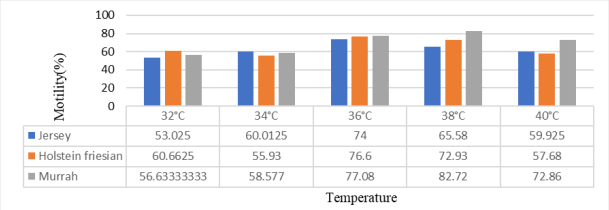

Fig. 1. Effect of temperature on spermatozoa motility of different breed

This study revealed that spermatozoa motility of jersey spermatozoa thawed at 36°C for 30 secs is 74%, (Fig: 1) which is greater than the result shown by Sabés-Alsina *et al*., (2017). The motility of HF spermatozoa, maximum at thawing temperature 36°C is 76.6% (Fig: 1), Closer to the finding of Malik *et al*., (2015). Because there were more live spermatozoa in an HF ejaculate, the percentage of total and progressively motile spermatozoa was greater in HF semen (Hoflack *et al*., 2006). The motility of Murrah spermatozoa thawed at 38°C is 82.72% (Fig: 1). greater than post-thaw motility reported by Pathak *et al*., (2020) at 37°C. The motility of sperm cells of the Murrah bull is higher at 38°C, this might be due to the genetic potency of the Murrah breed to resist slightly higher temperatures than other breeds, native to cold ecological zone.

Table I. Overall results of treatment with mean and SEM

Temperature	Breed	Motility (%)	Progressive M.	Fast M.	Slow M.	Immotile (%)
32°C	Jersey	53.02±6.73	26.42±5.57	6.175±1.30	18.79±5.37	51.40±8.74
	HF	60.67±6.31	32.47±6.31	8.40±1.80	24.02±5.12	39.88±3.82
	Murrah	56.63±3.93	33.26±3.06	4.59±3.06	31.32±2.77	43.28±3.94
34°C	Jersey	60.01±3.81	34.31±4.53	7.10±2.35	25.73±4.17	39.99±3.81
	HF	55.93±6.31	31.80±3.81	8.27±3.01	23.09±2.86	42.98±6.13
	Murrah	58.58±4.34	38.15±5.63	4.92±1.73	30.61±4.78	42.11±4.46
36°C	Jersey	74.33±1.40	52.41±2.97	12.71±2.04	19.51±3.70	25.73±1.37
	HF	76.60±2.28	56.29±4.65	18.43±1.63	17.41±4.02	23.04±2.28
	Murrah	77.09±1.59	54.41±2.66	7.01±0.99	28.77±2.14	22.09±1.58
38°C	Jersey	65.59±3.17	36.22±3.58	6.04±1.70	27.8±3.43	34.75±3.67
	HF	72.93±2.10	43.36±3.83	8.39±1.92	34.23±2.72	30.84±2.25
	Murrah	82.72±4.03	63.94±6.37	12.59±2.74	26.32±4.72	17.27±4.03
40°C	Jersey	59.92±3.58	34.49±4.05	9.60±1.12	24.46±4.06	44.57±5.41
	Holstein	57.69±2.28	28.11±2.32	7.42±0.99	20.55±2.71	42.26±2.31
	Murrah	72.87±4.00	53.70±6.02	7.61±2.61	45.4±4.722	27.11±3.99

The results are expressed in mean ± standard error of mean (SEM) M. stands for Motility
Motility is subdivided into progressive motility, Fast motility & Slow motility.

Effects of thawing temperature on spermatozoa progressive motility factor of different bull

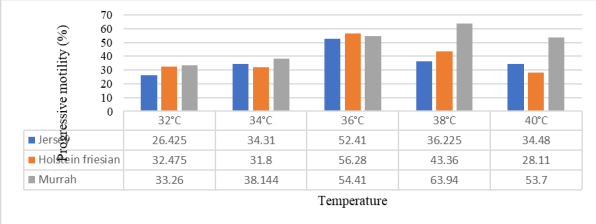

Fig. 2. Effect of temperature on spermatozoa progressive motility of different breed.

The graph shows that Jersey spermatozoa had maximum progressive motility (52.41%) (Fig: 2) at 36°C, lower than Li *et al*. (2016). HF spermatozoa peaked at 36°C (56.28%) (Fig: 2), higher than Zenteno *et al*. (2023), while Murrah showed the highest motility (63.94%) (Fig: 2) at 38°C, greater than finding of Sinha *et al*. (2021), likely due to their superior thermo resistance.

Sperm motility across varying thawing temperatures

Effects of thawing temperature on spermatozoa fast motility factor of different bull

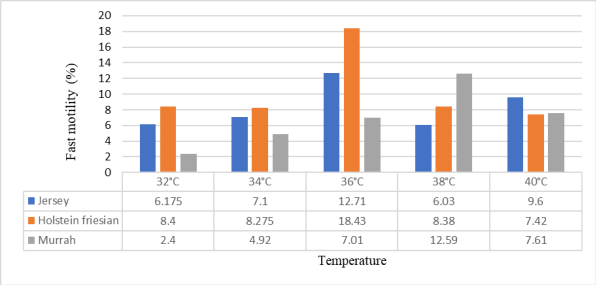

Fig. 3. Effect of temperature on spermatozoa fast progressive motility of different breed

The graph shows maximum spermatozoa fast motility at 36°C for Jersey (12.71%) (Fig: 3), similar to Fiaz *et al*. (2009) and Holstein Friesian (18.43%) (Fig: 3), aligning with Hoflack *et al*. (2006), while Murrah peaks at 38°C (12.59%) (Fig: 3), similar to Saini *et al*. (2018). Freezing and thawing cause oxidative stress, DNA damage, and reduced sperm viability Varghese *et al*. (2016).

Effects of thawing temperature on spermatozoa slow motility factor of different bull

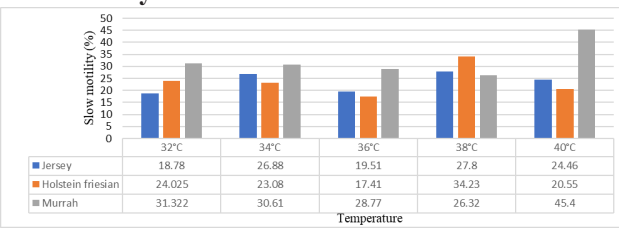

Fig. 4. Effect of temperature on spermatozoa slow progressive motility of different breed

The graph shows maximum spermatozoa slow motility at 36°C for Jersey (19.51%)(Fig:4) and HF (17.41%)(Fig:4), lower than (Iwaid Al-Badry et.al 2012), while Murrah peaks at 38°C (26.3%)(Fig:4), higher than Rastegarnia *et al*. (2013). Cooling-freezing and thawing cause variations due to their lethal effects (Kumar *et al*. 2015).

Effects of thawing temperature on spermatozoa immotile factor of different bulls

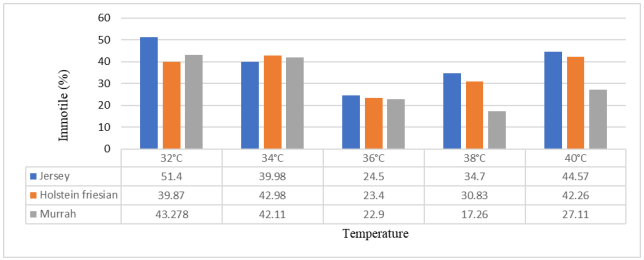

Fig. 5. Effect of temperature on spermatozoa immotile of different breed

The findings show minimum immotile spermatozoa at 36°C for Jersey (24.5%) (Fig:5), supporting Rastegarnia *et al*. (2013) and HF (23.4%) (Fig:5), while Murrah was lowest at 38°C (17.26%) (Fig:5), smaller than Pathak *et al*. (2020). Freezing thawing alters sperm motion and raises intracellular calcium, enhancing lateral head movement and circular motility Rastegarnia *et al*. (2013).

Comparison between treatment groups:

Table 2: Overall effect of thawing temperature on sperm motility

	Thawing temperature				
	32°C	34°C	36.5°C	38°C	40°C
Motility	56.77^{c}	58.13^{c}	76.05^{a}	74.19^{a}	63.87^{b}
Progressive Motility	30.82^{e}	34.89^{d}	54.37^{a}	48.48^{b}	39.36^{c}
Fast Motility	6.316^{c}	6.692^{c}	12.49^{a}	9.15^{b}	8.19^{b}
Slow Motility	24.98^{e}	27.01^{d}	41.81^{a}	38.33^{b}	30.75^{c}
Immotile	44.79^{a}	41.712^{b}	23.97^{e}	27.20^{d}	37.549^{c}

Different letters (a,b,c,d,e) in rows denote statistical difference ($p<0.05$)

The thawing temperature of different breeds reacts differently at different temperatures. The thawing of jersey and Holstein Friesian are best at 36°C but Murrah breed reacts differently than another comparative breed. Although initial motility is high at 38°C, thawing at higher temperatures increases motility and results in energy drainage and exhaust soon after a few durations. This reduces the viability of sperm cells for a long duration and sperm become inactive devoid of motion (Varghese *et al*. 2016). Higher total and progressive motility fresh semen samples are particularly susceptible to cryodamage (Muiño-Blanco *et al*. 2008).

Conclusion

The percentage of motility, progressive motility, and fast motility significantly increased in all breeds. While slow motility and immotile percentage decrease by thawing at 36˚C in 30 seconds in Jersey and Holstein Friesian and, At 38˚C in 30 seconds in Murrah species.

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Informed Consent Statement

Individuals' consent was received before data collection.

Data Availability Statement

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflict of interest

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